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Ozone in Relation to Storage of Apples

By H. A. SCHOMER, *associate physiologist*, and L. P. MCCOLLOCH, *associate plant pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration*[1]

CONTENTS

EVALUATION OF OZONE IN APPLE STORAGE

Ozone is generally used as a supplement to refrigeration in the commercial storage of eggs, because it is a deodorizer and a deterrent to surface molds which develop in the high humidities required. It is not widely used in the cold storage of apples and other fruits, but a few commercial storages employ it regularly.

The properties of ozone that make it desirable are its powerful oxidizing action, its pleasant odor in very dilute concentrations, and

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its harmless decomposition product, oxygen. It is widely used as a deodorizing agent because of its ability to oxidize many objectionable odors and gases into nonobjectionable products. Air containing 0.01 to 0.04 p. p. m. (parts per million) of ozone has a pleasant odor; that containing 2 to 3 p. p. m. is not unpleasant, but it may be irritating to the throat and may cause headache and nausea if exposure to it is prolonged. High concentrations have a strong, unpleasant odor and may cause headache and nausea when the exposure is short. Therefore, if concentrations of ozone as high as 2 to 3 p. p. m. are maintained, the generators are operated only when there are no workmen continuously in the room. The gas is unstable and decomposes rapidly; its disappearance under storage conditions results from a monomolecular decomposition by oxidation as demonstrated by Ewell (*5*),[2] who used the following monomolecular equations:

$$\text{Velocity of decomposition} = u = k\ (c - x)$$

$$k = \frac{2.3}{t} \log \frac{c}{c - x}$$

Ewell (*5*) found that the value for k in the equation was 0.0205 in an empty chamber in which he later placed apples. In the empty chamber the concentration of ozone was reduced from 1 to 0.54 p. p. m. after one-half hour. In the presence of apples the value for k was 0.051 and the ozone concentration was reduced from 1 to 0.21 p. p. m. after one-half hour, showing that the apples would absorb 0.33 p. p. m. in one-half hour and 0.66 p. p. m. in 1 hour. This finding indicates that the material of the exposed apple surfaces is readily oxidizable. The rate of ozone decomposition diminishes with the time of exposure, but removal of the apples to fresh air restores the higher absorption power.

A number of investigators (*6*, *10*, *19*, *21*, *22*, *23*, *24*) have recommended the use of ozone to reduce fruit decay and extend the storage period. Others (*1*, *20*), however, have reported that it has little or no effect on fruit decay or that more decay appeared in apples in ozonized than in normal atmospheres. Because of these divergent findings the United States Department of Agriculture is frequently asked about the advisability of using ozone. The studies reported in this circular were therefore undertaken to determine the effects of measured concentrations of ozone on the keeping qualities and appearance of apples and on certain apple pathogens.

During the first storage season (1941–42) of the experiment the ozone generator was operated 1 to 2 hours daily for 5 months, and the concentration of ozone in the storage atmosphere at the time of maximum ozone content averaged 1.95 p. p. m. by volume. No reduction of fruit decay occurred at this concentration. Because of the failure of ozone to reduce decay the concentration was increased during the 1942–43 storage season and averaged 3.25 p. p. m. during the hours of ozone production for 7 months of storage. During this season the ozonizer was operated about 8 hours per day. As during the previous season, there was little or no reduction of decay.

Although decay of the fruit was not checked during either storage season, air-borne fungus spores were killed by continuous exposure to

[2] Italic numbers in parentheses refer to Literature Cited, p. 23.

the ozonized atmosphere, so that the viable spores occurring naturally in the atmosphere were reduced to an insignificant number. Accompanying this lethal effect was the absence of mold development on the surfaces of packages and on storage-room walls. Boxes became moldy in the humid storage room in which the atmosphere was not ozonized (check lot), but no mold appeared on boxes in the ozonized atmosphere.

This effect of ozone makes it highly desirable in egg storage, for which a very high humidity is desired. Molds develop readily on the surfaces of the crates and of the eggs under such conditions, but ozone retards the mold development.

In spite of its control of surface mold, however, ozone failed to kill fungus colonies on the surface of packages when they had become established before ozonization. Although these colonies appeared to be dead and were inconspicuous as a result of ozonization, they were still viable after several months' exposure to the ozonized atmosphere.

Furthermore, exposure of apples for an entire storage season of 7 months to an atmosphere containing 3.25 p. p. m. of ozone failed to check decay. In a number of instances infection of inoculated fruit was even greater in the ozonized than in the nonozonized lots. This was thought to be due to further injury of the wounded tissue by ozone, which predisposed it to fungus invasion. It appears that the rapid interaction of ozone with organic matter prevents its penetration below the surface of the substrate, so that fungi protected by fruit tissue, as in fruit inoculated naturally or artificially, are not killed by ozone although their rate of growth is retarded.

Since apple scald is caused by volatile emanations from the fruit and ozone oxidizes organic substances with unsaturated bonds, such as ethylene, observations were made on the effect of ozone on scald development in Arkansas (Mammoth Black Twig) apples, a variety very susceptible to the disorder. Scald development was reduced appreciably by the ozone, but not sufficiently to afford satisfactory control.

At the concentration of 3.25 p. p. m., injury due to ozone occurred on every variety in storage. It appeared as brown sunken areas at the lenticels, the intensity varying with the variety. Golden Delicious was the most sensitive of the varieties used in the experiment, whereas the injuries occurring on Winesap and Delicious were hardly objectionable. Rome Beauty apples were injured by a single exposure when they were in a storage room in which the ozonizer was operated 2¾ hours and produced an ozone concentration of 33.9 p. p. m. The apples were not removed from the room after the generator was turned off, and the injury appeared within a few days.

In addition to injury of lenticel tissue, there were other serious effects of extended exposure to 3.25 p. p. m. of ozone. The surface (skin) of some varieties became sticky and varnishlike in appearance. The flavor of the fruit (except Golden Delicious) was impaired, the extent of the off-flavor varying with the variety. Previously, in a survey of several large commercial cold storages in the East, there were found a number of operators who refused to use ozone in apple storage for fear of impairing the flavor of the fruit, but no reference was found in the literature to support their contention. During this study the objectionable effects on flavor resulted from 7 months

of storage in an atmosphere containing 3.25 p. p. m. of ozone for several hours per day, but none of them occurred in an atmosphere containing 1.95 p. p. m. during 5 months of storage.

Ozone appeared to have no effect on major physiological activities of apples. This was demonstrated by the fact that no difference could be found in the maturity of ozonized and nonozonized fruit at any time during the storage period as measured by pressure tests, composition of internal atmosphere, pH, and total acidity.

The experimental data upon which these conclusions are based are given in the following pages.

MATERIALS AND METHODS

Storage Facilities and Fruit

During the 1941–42 and 1942–43 storage seasons the effects of ozone on the keeping qualities of apples were studied. Each of the two storage rooms used had a capacity of 825 cubic feet, or approximately half a carload of apples; in both seasons both rooms were filled to capacity. During storage the temperature was 31° F. and the relative humidity was approximately 88 percent. One room had a normal storage atmosphere and the other an ozonized atmosphere; the air in both rooms was continuously circulated by 16-inch electric fans. The ozonizer was operated daily except Sundays beginning October 31. During 1941–42 it was operated only 1 to 2 hours daily, since it had been reported (*24*) that ozone was as effective when applied once daily as when applied continuously; but during 1942–43 it was run about 8 hours daily. The average ozone concentration reached during the first season was 1.95 p. p. m. by volume. Because of its failure to reduce decay, the ozone concentration was increased during 1942–43; and the average of the daily concentrations measured after the ozone had reached a constant level was 3.25 p. p. m.

The apple varieties stored were Delicious, Golden Delicious, York Imperial, Rome Beauty, Winesap, and Arkansas. The special lots for periodical examination and for inoculation were composed of carefully selected fruit. Duplicate lots were placed in each storage room. The rest of each room was filled with orchard-run fruit.

Ozonizer

The ozone generator, which was constructed and assembled in the laboratory, was essentially like that described by Goss and Phillips (*13*). It consisted of three Berthelot tubes made of Pyrex tubing (fig. 1). The tubes were partially immersed in water in a large battery jar, 9 by 12 by 17 inches; they were connected in series by means of oil seals (fig. 2). Sufficient alcohol was added to the water to prevent freezing when the ozonizer was used in storage rooms held at 31° F. A coil of copper tubing was fitted inside the bottom of the jar, the two ends of the tubing being bent upright to extend parallel above the top. This copper tubing served as one electrical terminal. A cover of asbestos fiberboard was fitted over the top of the jar to accommodate and hold in place the ozonizing tubes and the copper tubing.

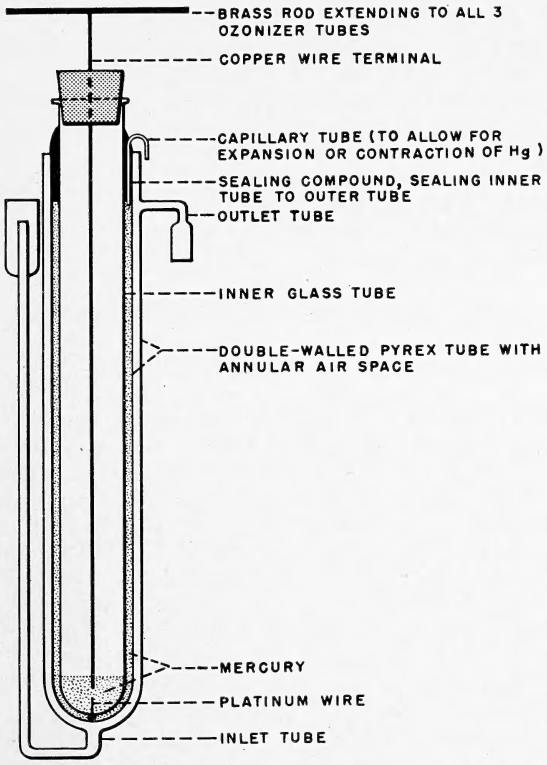

FIGURE 1.—Longitudinal section of an ozonizer tube.

The air-inlet and air-outlet tubes of the ozonizer were equipped with the inner parts of standard taper joints so that rubber tubing was not used for these connections. Moisture-free air or oxygen was forced through the ozonizer at the desired rate. Glass tubing was used for all connections and air lines.

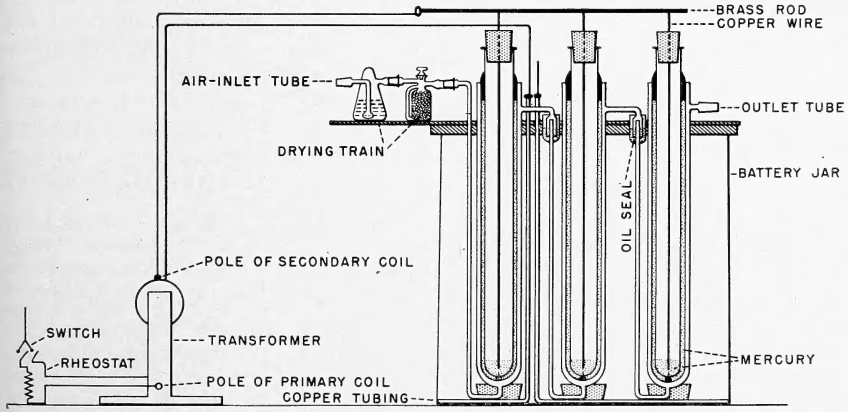

FIGURE 2.—Diagram of ozonizer assembly.

The two wires of the 110-volt alternating-current line were attached to the two poles of the primary coil of a 25,000-volt transformer. An electric switch, a rheostat, and a voltmeter were placed in this line One of the poles of the secondary coil was connected to the brass rod leading to the three copper wires of the ozonizer tubes; the other pole was connected to the copper coil immersed in the water of the battery jar. The voltage during operation of the ozonizer was varied from 70 to 90 in the primary coil as desired.

During operation the electric discharge occurred in the annular spaces between the mercury cylinders and the outer cylinders of the Berthelot tubes; in the dark it appeared as a continuous glow in the tubes. No opportunity was afforded for sparking between electrodes such as often occurs in ozone generators which employ metallic electrodes separated by insufficient dielectrics. As a consequence, the possibility of the formation of oxides of nitrogen, which also have bactericidal properties (*26*), was obviated; and no trace of them could be found in the storage room.

Method of Analysis

Sampling of atmosphere.—A Pyrex tube [3] was extended into the center of the storage room through a small aperture in the wall. The atmosphere from the room was drawn through a gas-washing bottle by means of suction, the volume of air being accurately measured with a precision wet-test gas meter placed between the gas-absorption bottle and the suction pump. Connections to the gas-absorption bottle were made by means of Pyrex standard taper joints.

Ozone determination.—Thorp's modification (*25*) of the starch-iodide method was used for the ozone analysis.

Solutions needed.—The solutions used consisted of (1) a 2 N solution of potassium iodide; (2) a buffer solution consisting of 5 grams of aluminum chloride hexahydrate and 1 gram of ammonium chloride made up to 1 liter with distilled water; (3) a 0.01 N solution of sodium thiosulfate; and (4) a starch solution as an indicator.

Procedure.—To 100 milliliters of the 2 N potassium iodide solution 5 milliliters of the buffer solution of aluminum chloride hexahydrate and ammonium chloride was added, and 100 milliliters of the solution was placed in the gas-washing bottle. The ozonized air to be analyzed was drawn through the solution until a yellow color was obtained. The volume of air was accurately read from the gas meter, and the free iodine that had been liberated from the potassium iodide by the ozone was titrated with a 0.01 N solution of sodium thiosulfate; starch solution was used as the indicator. An equal volume of air free from ozone drawn through a second gas-washing bottle of

[3] Glass is inert to the action of ozone; because of this property it was used wherever contact with ozonized atmospheres was necessary. Rubber tubing rapidly disintegrates in the presence of ozone and cannot be used in any operation when it is in contact with that gas. Because the question arose whether it would be permissible to sample an ozonized atmosphere through a copper tube, a series of determinations was made to compare the analytical results of duplicate samples of air drawn through copper and Pyrex tubing. No differences were obtained. Consequently, it was concluded that copper tubing of the comparatively short length required for sampling ozonized atmospheres from storage rooms would be satisfactory.

potassium iodide solution constituted the check. The difference in the titration values was used for determining the ozone concentration in parts per million. Since 1 milliliter of 0.01 N solution of sodium thiosulfate equals 0.112 milliliter of ozone the formula $\frac{T \times 112}{S}$ = parts per million of ozone (based on volume) was used. In this formula T equals number of milliliters of sodium thiosulfate required for titration and S equals liters of gas sample used in analysis after correction to standard conditions.

EFFECT OF OZONE ON FUNGUS SPORES

Spores on Glass-Slide Cultures

Spores of *Botrytis cinerea* Fr., which causes gray mold rot, and of *Penicillium expansum* (Lk.) emend. Thom, which causes blue mold rot, were distributed evenly over a gelatin film on microscope slides. A number of such slides were exposed daily for 1-hour periods to the atmospheres of the ozonized and the nonozonized room. Each day three slides from each room were withdrawn after exposure, placed in sterile petri dishes, and flooded with a nutrient agar. The remaining slides were placed in moist chambers and stored at 31° F. until the following day's exposure. The petri dishes were held at room temperature, and germination counts on a number of microscope fields were made at definite intervals to determine the effectiveness of the ozone treatments.

An atmosphere containing 1.95 p. p. m. of ozone caused a definite reduction in germination of the spores. A single 1-hour exposure reduced germination of spores of *Botrytis cinerea;* it was 18.8 percent in the ozonized room (table 1) as compared with 62.8 percent in

Table 1.—*Effect of exposure of glass-slide cultures of Botrytis cinerea and Penicillium expansum to an atmosphere containing 1.95 p. p. m. of ozone on spore germination, 1941–42*

Fungus and number of 1-hour exposures	Ozonized room			Nonozonized room		
	Fields examined	Spores counted	Spores germinated	Fields examined	Spores counted	Spores germinated
Botrytis cinerea:	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
1	30	1, 225	18. 8	30	1, 101	62. 8
2	32	1, 344	6. 8	30	835	55. 4
3	30	699	1. 3	10	163	58. 9
4 [1]	30	1, 056	1. 6	12	124	98. 4
5	10	463	0	10	269	24. 2
Penicillium expansum:						
1	60	2, 501	59. 6	30	1, 656	81. 8
2	120	6, 223	2. 1	30	1, 888	81. 8
3	30	1, 855	16. 2	30	1, 594	55. 0
4	10	465	0	10	650	48. 0

[1] Only 1 plate showed any germination (4.8 percent).

the nonozonized one. After four 1-hour exposures in 4 days spore germination in the ozonized room was 1.6 percent as contrasted with 98.4 percent in the nonozonized one. Generally the exposures were stopped after the fourth day because sufficient desiccation of the film had occurred to cause a reduction in germination. The slides were examined daily for spore germination; when growth on the nonozonized slides had advanced so far that counts could no longer be made, the slides were discarded. However, in some instances spores on the ozonized slides that had appeared dead up to this time germinated later. It is therefore concluded that ozone has a definite retarding effect upon germination as well as a lethal effect. As would be expected, scattered single spores were more sensitive to ozone than were heavy clumps of them.

Similar results were obtained with *Penicillium expansum*. After four 1-hour exposures in 4 days, none of the ozonized spores and 48 percent of the nonozonized spores germinated.

Natural Spore Load of Storage-Room Air

After 4 months of storage a survey was made of the natural spore loads of the air in the ozonized (3.25 p. p. m.) and the nonozonized room. To assure complete spore circulation, in addition to the regular storage-room fan, a 20-inch propeller-type fan that delivered 3,400 cubic feet per minute was operated 1 hour before and during the exposure period. Petri dishes containing nutrient agar were exposed for 30 minutes. After several days at room temperature the colonies were counted. There was a great reduction in the number of viable spores in the ozonized room (fig. 3); averages of 785 fungus colonies per plate in the nonozonized room and of only 6 colonies per plate in the ozonized one were obtained. Most of the fungus colonies were *Penicillium*.

Surveys were also made in commercial cold-storage plants in which ozone was being used. In one plant sterile petri dishes were exposed for 30 minutes at various locations in egg-storage rooms in which the ozone concentration averaged about 1.8 p. p. m. In these rooms the air was circulated by blowers and the relative humidity at the time of exposure was 80 percent. This concentration of ozone was relatively effective in reducing the number of air-borne micro-organisms. Averages of 5.2 fungus colonies per plate exposed in the ozonized room and of 28.4 colonies per plate in the nonozonized room were found (table 2).

In another plant in which ozone was being used in a number of apple-storage rooms, its concentration was held at 0.7 to 1.0 p. p. m. Forced-air circulation was not employed in this plant, and the relative humidity was 85 percent. For experimental purposes ozone was generated for a single 24-hour period in one apple-storage room which had not been previously treated with ozone. The concentration of ozone obtained was comparatively low, reaching only 0.37 p. p. m. Exposure of plates containing sterile nutrient agar for 30 minutes revealed that there was little or no control of micro-organisms under commercial conditions from such low concentrations of ozone (table 3).

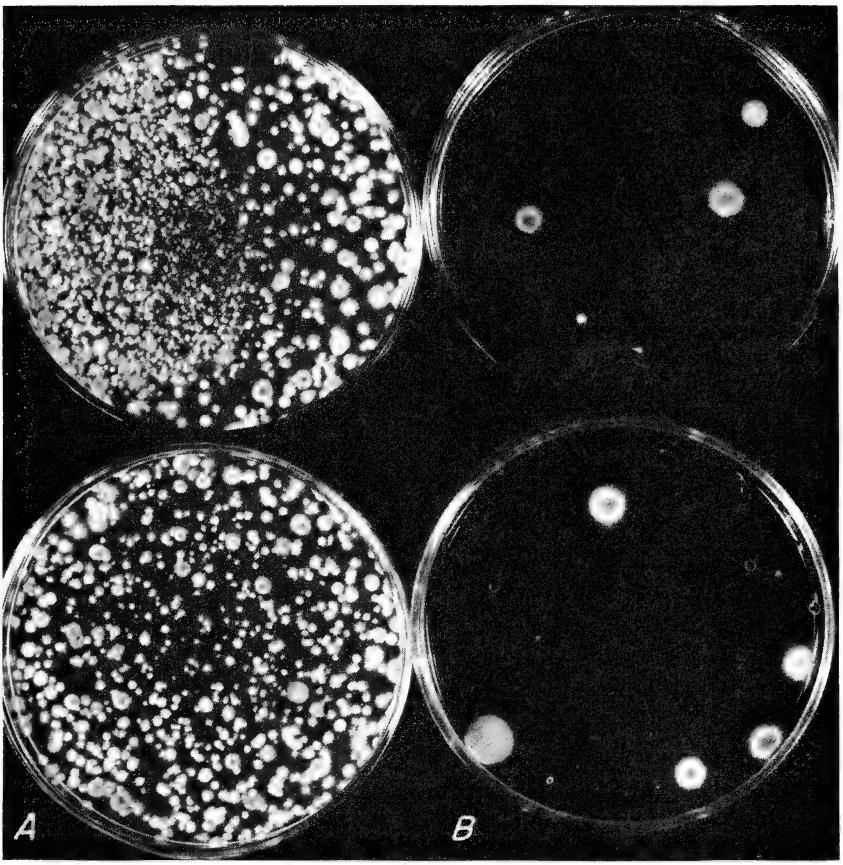

FIGURE 3.—Effect of ozone on the natural spore load of air in storage rooms filled with apples at 31° F.: *A*, *Penicillium* colonies on plates exposed for 30 minutes in a room without ozone and with a blower operating 1 hour before and during exposure; *B*, colonies on plates exposed in a room in which a concentration of 3.25 p. p. m. of ozone was generated daily for 4 months of storage and a blower was operated as in *A*.

TABLE 2.—*Effect of 1.8 p. p. m. of ozone in an egg-storage room of a commercial cold-storage plant on the number of colonies per plate exposed for 30 minutes*

Kind of colony	Colonies per plate exposed in—	
	Ozonized room	Nonozonized room
	Number	*Number*
Penicillium spp. and other fungi [1]	5. 2	28. 4
Bacteria	2. 4	18. 8

[1] The majority of the colonies were *Penicillium* (4.8 and 27.8, respectively).

TABLE 3.—*Effect of 0.37 to 1.0 p. p. m. of ozone in apple-storage rooms of a commercial cold-storage plant on the number of colonies per plate exposed for 30 minutes*

Kind of colony	Colonies per plate exposed in—		
	Room containing 0.7–1.0 p. p. m. of ozone (continuous)	Room containing 0.37 p. p. m. of ozone (24 hours)	Nonozonized room
	Number	*Number*	*Number*
Penicillium spp. and other fungi [1]	35. 0	30. 4	43. 5
Bacteria	. 3	1. 0	. 8

[1] The majority of the colonies were *Penicillium* (34.5, 29.7, and 43.0, respectively).

Further evidence of the ineffectiveness of ozone as a fumigant was reported by Smock and Watson (*24*), who found that 0.6 p. p. m. killed spores of *Penicillium expansum* and *Sclerotinia fructicola* (Wint.) Rehm equally well when they were wet or dry if they were not in clumps, but that if the spores were protected by moist surfaces of apple flesh or other organic protectants the ozone may have no effect on germination. Hartman (*15*) likewise stated that ozone in a concentration of 0.5 to 1.0 p. p. m. was decidedly inhibitory toward nearly all forms of micro-organisms, but that it was not germicidal until 13 to 14 mg. per liter (6,500 p. p. m.) was reached. He stressed the need for differentiating an inhibitory from a germicidal concentration. Elford and Van den Ende (*4*) found that ozone in the very dilute concentration of 0.04 p. p. m. was able to inactivate certain bacteria when they were present as unprotected singleton aerosol particles, but that when such bacteria were covered by a protective coating, as in a spray (sneeze particles), this concentration was without effect.

These results substantiate the earlier observation that ozone in very dilute concentrations has an inhibitory or even lethal effect on micro-organisms if they occur singly and unprotected. However, if they occur in clumps or are protected by surrounding films, the ozone is relatively ineffective.

Spore Load Introduced in Air of Empty Storage Room

At the end of the storage season, after the apples had been removed from the two test rooms, a series of experiments was conducted to determine the effectiveness of high concentrations of ozone in destroying fungus spores in empty storage rooms. To insure a heavy spore load, spores of *Penicillium expansum* were introduced into the room at the beginning of each experiment. Petri dishes containing sterile nutrient agar were exposed for 30 minutes before the introduction of ozone and at intervals during the ozonization as a means of determining its effectiveness in killing spores. Air movement was moderate during the experiment.

The effects of intermittent high concentrations of ozone are illustrated in figure 4. Large percentages of the spores were killed after

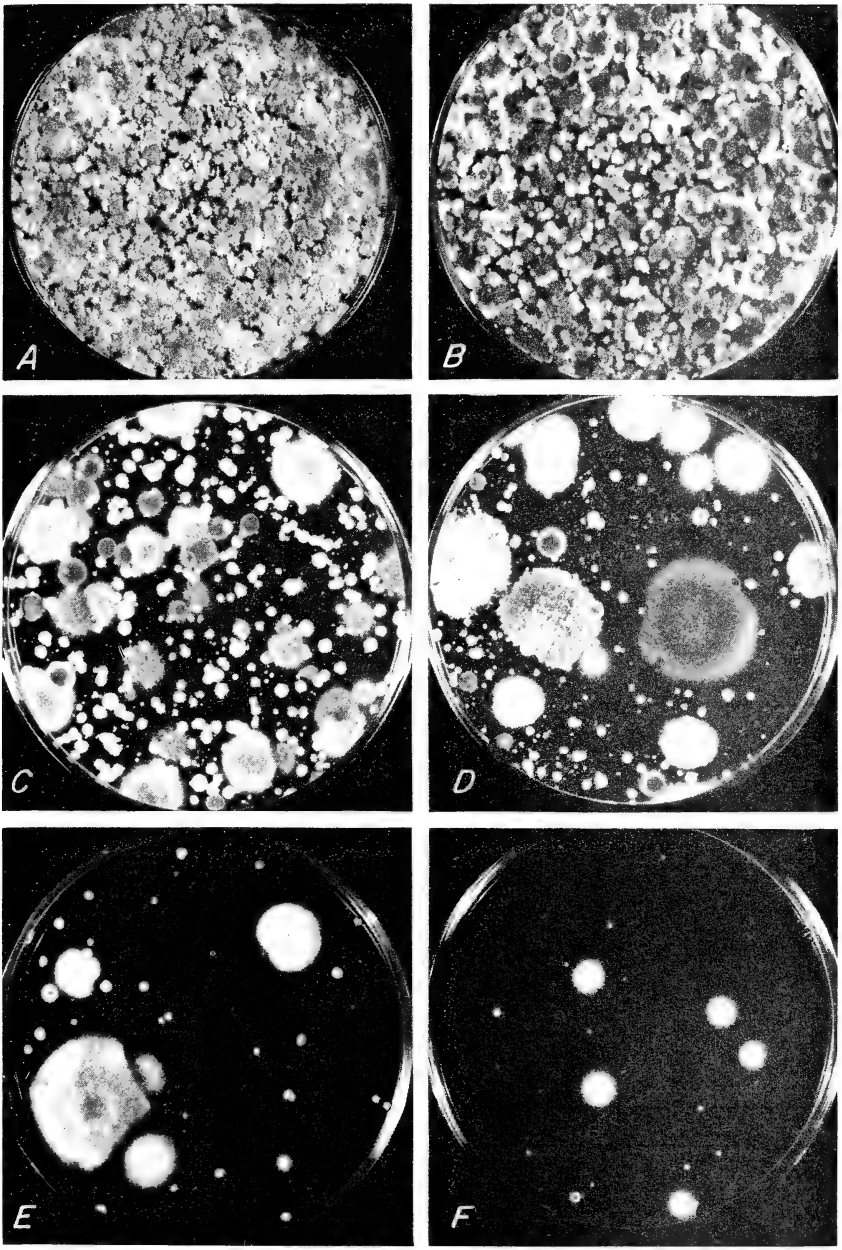

FIGURE 4.—Effect of intermittent high concentrations of ozone in the air on the spore population of *Penicillium expansum* introduced into a storage room as indicated by the colonies developing on nutrient-agar plates exposed for 30 minutes: *A*, Plate exposed 4 hours after spores had been introduced; *B*, plate exposed after the room had been ozonized for 2¾ hours and the ozone concentration had reached 33.9 p. p. m.; *C*, plate exposed 16 hours after that shown in *B* without any addition of ozone; *D* and *E*, plates exposed after 4½ and 6½ hours, respectively, of additional ozonization during which the concentration of ozone reached 50.4 p. p. m.; *F*, plate exposed 16 hours after that shown in *E* without any addition of ozone.

relatively short exposures. With the wide range of ozone concentrations used, considerable variation was obtained in the rates of sterilization. The immediate effectiveness of ozone as a fungicide appeared to be closely related to the concentration used. The high concentrations shown in table 4 and in experiment 1 (table 5) killed spores more rap-

TABLE 4.—*Effect of intermittent high concentrations of ozone in the air on the spore population of Penicillium expansum as indicated by the colonies developing on nutrient-agar plates exposed for 30 minutes*

[Ozonizer operated daily from 8:30 a. m. to 4 p. m.; 1,184 colonies developed per plate exposed to determine concentration of spores before ozonization (last column of table based on this value)]

Ozonization period	Ozone concentration at time of exposure	Relative colony development
	P. p. m.	
0 hour	0	100
2¾ hours	33. 9	77
19 hours [1]	0	34
24 hours	50. 4	10
26 hours	50. 4	6
42 hours [1]	0	2

[1] Plate exposure after ozonizer had been idle 16 hours.

TABLE 5.—*Effect of continuous high concentrations of ozone in the air on the spore population of Penicillium expansum as indicated by the colonies developing on nutrient-agar plates exposed for 30 minutes*

[Ozonizer operated continuously for duration of experiments; 363 colonies in experiment 1 and 498 in experiment 2 developed per plate exposed to determine concentration of spores before ozonization (last column of table based on these values)]

Experiment and ozonization period	Ozone concentration at time of exposure	Relative colony development
Experiment 1:	*P. p. m.*	
0 hour	0	100
5 hours	28. 0	5. 7
7½ hours	43. 6	3. 6
19 hours	21. 9	7. 9
43 hours [1]	0	7. 6
Experiment 2:		
0 hour	0	100
48 hours	20. 7	37. 9
72 hours	30. 2	4. 4
89 hours	20. 1	7. 4
96 hours	20. 8	1. 2

[1] Plate exposure after ozonizer had been idle 8 hours.

idly than the lower concentrations shown in experiment 2 (table 5), but the spore viability was very effectively reduced in all the tests when the exposure was long enough. This finding substantiates the conclusion of Ewell (*8*) that killing increases with concentration of the ozone as well as with the time of exposure.

Mold on Packages

During the two storage seasons observations were made on mold development on packages and on the storage-room walls. Veneer boxes which had been wet before being placed in storage were very susceptible to mold and in the control room many became covered with extensive growth (fig. 5, *B*). Similar boxes in the ozonized room appeared strikingly clean and free from mold (fig. 5, *A*). However,

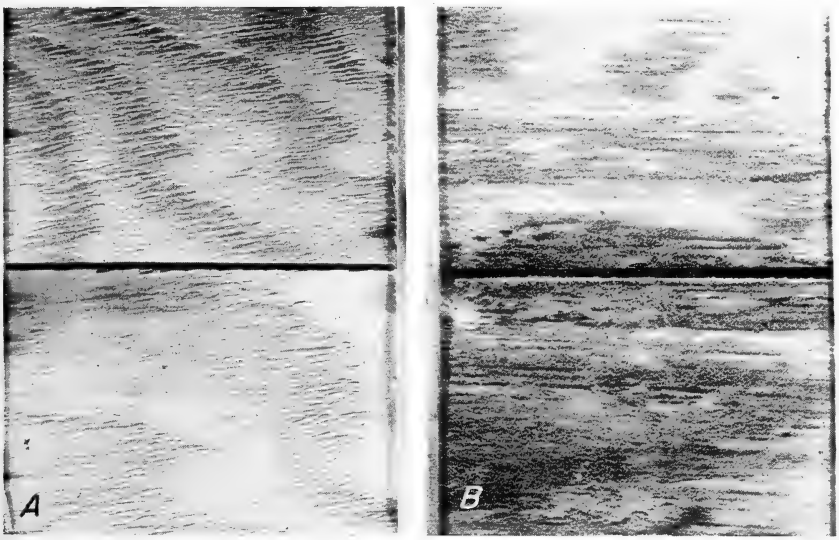

FIGURE 5.—Apple boxes stored for 5 months at 31° F.: *A*, Exposed to 3.25 p. p. m. of ozone; *B*, not exposed to ozone and showing abundant mold.

upon examination after 5 months in storage a few small, black, inconspicuous mold colonies were found on some of the ozonized boxes. To determine the viability of these established mold colonies, bits of the mold were transferred to petri dishes containing nutrient agar. Colonies of *Penicillium* grew from the transplantations, showing that daily ozonization with an average concentration of 3.25 p. p. m. for a period of 5 months had failed to kill the fungus.

Two boxes from the control room having extensive mold growth were placed in the ozonized room. After 16 weeks in the ozonized atmosphere, the mold was shrunk and dried and had become relatively inconspicuous. Cultures demonstrated, however, that the mold was still viable.

These results are in agreement with those of Klotz (*20*), who found that ozone only partially inhibited the germination and growth on agar of the blue and green molds that cause penicillium rots of citrus

fruits. When the cultures were removed from the ozonized chamber after 3 weeks' exposure, they resumed their usual rapid rate of growth. Haines (*14*) found that ozone in concentrations greater than 200 p. p. m. was needed to sterilize bacteria in a vegetative state on nutrient agar. Thus, it is concluded that once the growth of these micro-organisms has become established, high concentrations of ozone are required to kill them.

EFFECT OF OZONE ON THE DEVELOPMENT OF FRUIT DECAY

In the course of harvesting and packing apples, the fruit is exposed to inoculation by various fungi, some of which cause decay. An important phase of the present investigation, therefore, was to determine the effect of ozone on the infection and subsequent development of decay in apples. In order to obtain comparative data, large numbers of apples were inoculated artificially with *Penicillium expansum* and *Phialophora malorum* (Kidd and Beaum.) McColloch and subsequent decay development was observed. The natural decay development on the regular apple pack was also observed.

Fruit Inoculated With Penicillium Expansum

Sound apples were wounded on two opposite sides or on three areas by means of a cork with four pins protruding one-fourth of an inch. The apples were then dipped in an aqueous spore suspension to which a small amount of a commercial wetting agent had been added. The spore suspension was made by scrubbing four agar slants of *Penicillium expansum* into 6 liters of tap water. After being dipped the fruit was allowed to dry and was placed in the storage rooms in open, paper-lined boxes.

Ozonization failed to prevent germination of spores lodged in the wounds and to stop subsequent decay, although at a concentration of 3.25 p. p. m. ozone injured the fruit. As a matter of fact, in some instances a higher percentage of inoculated wounds developed into actively decaying areas on the ozonized fruit than on the nonozonized, as shown in table 6 for Rome Beauty apples. After 30 days of storage at 31° F. and 5 days at 70°, 90.6 percent of the inoculations on the ozonized (3.25 p. p. m.) fruit were actively decaying as compared with 62.5 percent of those on the nonozonized fruit. In another lot which was held for 63 days at 31° and for 3 days at 70°, 76 percent of the inoculations had developed into decayed areas on the ozonized fruit and 48.2 percent on the nonozonized fruit. Thus, it appears that instead of preventing penicillium decay ozone may stimulate its development. Similar results have been observed by other investigators (*1, 3, 18*), working with different micro-organisms. A possible explanation is that the wounded tissue was damaged further by the ozone, giving very favorable conditions for fungus invasion. Another explanation that has been given is acclimatization of the organism to ozone (*16, 18*). However, Ewell (*7*) was unable to confirm acclimatization of mold to ozone; and observations after a season of ozonization of the regular commercial pack do not support this contention.

TABLE 6.—*Effect of ozone on development of decay on wounded apples inoculated with spores of Penicillium expansum and subsequently stored in ozonized rooms at 31° F. for various periods*

[Final examination of fruit made after 1 to 5 days at 70° F. without ozone to simulate marketing period]

Variety and ozone treatment	Storage period at 31° F.	Subsequent period at 70° F.	Fruit in test	Inoculation wounds	Wounds infected		
					Decay		Total
					Normal	Retarded	
	Days	*Days*	*Number*	*Number*	*Number*	*Number*	*Percent*
Rome Beauty:							
3.25 p. p. m.	30	5	8	64	34	24	90. 6
0			8	64	33	7	62. 5
3.25 p. p. m.	60	0	566	4, 528	249	1, 016	27. 9
0			555	4, 440	332	151	10. 9
3.25 p. p. m.	61	1	200	1, 600	-------	-------	60. 6
0			191	1, 528	-------	-------	23. 9
3.25 p. p. m.	63	3	366	2, 928	2, 225	0	76. 0
0			364	2, 912	1, 405	0	48. 2
York Imperial:							
Experiment 1:							
1.95 p. p. m.	30	2	67	804	[1]	-------	93. 3
0			65	780		-------	82. 0
Experiment 2:							
3.25 p. p. m.	12	0	6	48		-------	83. 3
0			6	48		-------	95. 8
3.25 p. p. m.	20	0	6	48		-------	97. 9
0			6	48		-------	97. 9
3.25 p. p. m.	30	1	12	90	9	38	52. 2
0			12	96	72	15	90. 6
3.25 p. p. m.	30	3	12	90	38	48	95. 6
0			12	96	86	8	97. 9
3.25 p. p. m.	51	0	139	1, 112	448	308	68. 0
0			137	1, 096	827	83	83. 0
3.25 p. p. m.	52	1	139	1, 112	906	0	81. 5
0			137	1, 096	923	0	84. 2

[1] No segregations made.

The ozonized and nonozonized lots of York Imperial, a variety not as sensitive to ozone injury as Rome Beauty, exhibited no marked differences in decay development. As shown in table 6, 93.3 percent of the inoculated wounds on York Imperial apples stored for 30 days in an atmosphere containing 1.95 p. p. m. of ozone developed into active decay as compared with 82 percent of those on the nonozonized fruit. In another lot stored for 30 days at 31° F. in an ozone concentration of 3.25 p. p. m. and then for 3 days at 70° without ozone, 95.6 percent of the wounds on inoculated fruit developed into decays as compared with 97.9 percent of the wounds on the nonozonized fruit. When a third lot of this variety was held for 52 days at an ozone concentration of 3.25 p. p. m., 81.5 percent of the wounds developed into active decays as compared with 84.2 percent of the wounds on nonozonized fruit.

Although ozone did not check infection of wounds, it retarded enlargement of the lesions (fig. 6). In table 6 unusually small lesions

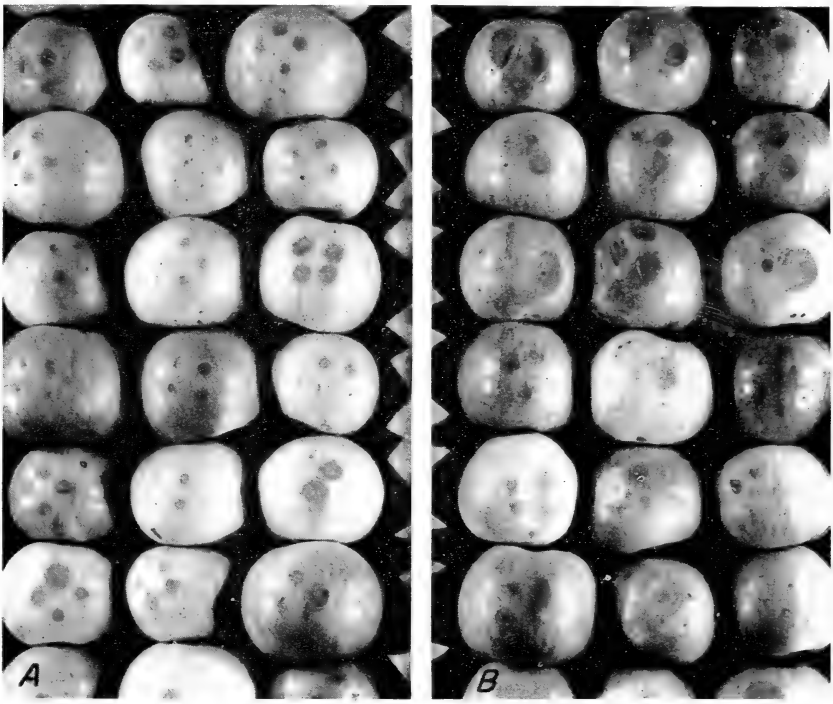

FIGURE 6.—Effect of ozone on blue mold decay on Rome Beauty apples inoculated with *Penicillium expansum* and stored for 60 days at 31° F.: *A*, In atmosphere containing 3.25 p. p. m. of ozone; *B*, in nonozonized atmosphere.

on both the ozonized and the nonozonized fruit are recorded as retarded. Obviously the small lesions on the nonozonized lots were due to factors other than ozone. After 51 days' storage at 31° F. ozonized York Imperial apples had 40.7 percent of the lesions retarded and the nonozonized ones 9.1 percent. Thus ozone was responsible for retarding growth in approximately 31.6 percent of the lesions. After 60 days at 31°, 80.3 percent of the lesions on ozonized Rome Beauty apples were retarded and 31.3 percent on the nonozonized ones; or 49 percent of the lesions were retarded by the ozone. Retardation of decay occurs only when ozone is present, however; after removal of the fruit from the ozonized atmosphere, the decay lesions increase in size as rapidly as do those on the nonozonized fruit. Although the retardation is considerable, the affected fruit is still unacceptable and so the retardation is not commercially important.

Fruit Inoculated With Phialophora Malorum

Sound apples of a uniform degree of maturity were selected for inoculation with *Phialophora malorum*, the fungus that causes the so-called side rot of apples. Fruit free from wounds was selected

because it was imperative that wound parasites such as blue mold should not get started.

The fruit was dipped into an aqueous spore suspension to which was added a small amount of a commercial wetting agent as a spreader. No effort was made to control the concentration of spores, but the inoculum was examined microscopically to make certain that there was an abundance of them. The fruit was allowed to dry and was then packed in boxes and stored in equal lots in the two test rooms.

Ozone failed to control side rot on the apples that had been inoculated with spores of *Phialophora*. In some instances more infections occurred on the ozonized fruit than on the nonozonized. This may have been due to lenticel injuries caused by the ozone.

A summary of the results of these tests is given in table 7.

TABLE 7.—*Effect of ozone on the development of decay on wounded apples inoculated with spores of Phialophora malorum and subsequently stored in ozonized rooms at 31° F. for various periods*

[Final examination made after 7 days at 70° F. without ozone]

Variety and ozone treatment	Storage period at 31° F.	Subsequent period at 70° F.	Fruit inoculated	Fruit infected	Lesions per fruit
Rome Beauty:	*Days*	*Days*	*Number*	*Percent*	*Number*
3.25 p. p. m.	130	7	61	68. 9	5. 6
0			65	72. 3	7. 1
York Imperial:					
3.25 p. p. m.	130	7	45	100. 0	32. 4
0			35	94. 3	19. 2
1.95 p. p. m.	90	7	45	22. 2	. 9
0			129	9. 3	. 2

FRUIT IN THE REGULAR PACK

All the test fruit was examined near the end of each storage season to determine the effect of ozone on development of decay in the regular pack. With one exception there was consistently less decay in the ozonized fruit than in the nonozonized, but the difference was very slight. This difference could be accounted for by the small number of infections that occurred after the apples were placed in the nonozonized storage. Evidence indicates that ozone kills unprotected spores in the air, but not those protected by wounded tissue or by the tissue of natural infection courts such as lenticels. Smock and Watson (*24*) reported that ozone may have no effect on spore germination if the spores are protected by moist surfaces of apple flesh or by other organic protectants. In an interesting experiment designed to test the penetrative power of ozone, Elford and Van den Ende (*4*) found that an ozone concentration of 330 p. p. m. achieved complete surface sterilization of serum agar inoculated with *Staphylococcus aureus* but that there was no appreciable penetration of disinfecting action below the surface. These findings emphasize the striking weakness of ozone as a fumigant; once an organism has become established so that any

part of it is protected by certain organic substances such as plant tissue, it will not be killed by tolerable concentrations of ozone.

The data for the fruit decay in the regular pack for the two storage seasons are summarized in table 8. There was no decay on the Golden Delicious test fruit removed from storage January 15, 1943, and none on the Arkansas test fruit removed from storage March 3 and April 28, 1943, or after 1 week at 70° F.

TABLE 8.—*Effect of ozone on development of decay in apples stored at 31° F.*

Experiment, variety, and ozone treatment	Fruit waxed or unwaxed	Fruit		Date examined	Fruit affected with—				
					Blue mold	Side rot	Gray mold	Miscellaneous decays	All decays
		Boxes	*Number*		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Experiment 1:									
Golden Delicious:									
1.95 p. p. m.	Unwaxed	5	696	Mar. 17	1.1	0	0	0	1.1
0	do	5	678	do	1.0	0	.4	.6	2.0
Delicious:									
1.95 p. p. m.	do	6	613	May 13	8.3	.3	0	3.1	11.7
0	do	6	658	do	5.0	.4	.6	3.8	9.8
York Imperial:									
1.95 p. p. m.	do	8	763	do	2.7	1.0	.2	3.0	6.9
0	do	6	546	do	2.7	0	.7	3.7	7.1
Winesap:									
1.95	do	6	870	do	0	0	0	0	0
0	do	6	875	do	0	0	.2	0	.2
Experiment 2:[1]									
Delicious:									
3.25 p. p. m.	do	5	534	Mar. 4	3.2	0	0	1.5	4.7
0	do	5	532	do.[1]	4.3	0	.9	.4	5.6
Winesap:									
3.25 p. p. m.	Waxed	4	636	May 24	.8	5.0	0	0	5.8
0	do	3	497	do	1.2	5.6	.6	0	7.4
3.25 p. p. m.	Unwaxed	2	306	do	0	1.3	.3	0	1.6
0	do	1	131	do	0	2.3	.7	.8	3.8
3.25 p. p. m.	Waxed	4	591	May 31 [2]	1.0	6.7	0	.2	7.9
0	do	3	452	do	.9	10.6	0	0	11.5
3.25 p. p. m.	Unwaxed	2	290	do	.3	3.4	0	0	3.7
0	do	1	123	do	0	7.3	0	0	7.3

[1] Golden Delicious apples were removed from storage Jan. 15, and Arkansas apples were examined Mar. 3 and Apr. 28 after 7 days at 70° F. No decay was found in either variety on these dates.

[2] After 1 week at 70° F.; percentages include decay present on May 24.

EFFECT OF OZONE ON DEVELOPMENT OF SCALD

The Arkansas variety was selected to determine the effects of ozone on scald development because of its extreme susceptibility to that disorder. The experimental fruit was sorted into highly colored (red) and green lots. Fruit with more than 50 percent of the surface green

in color was classified as green and constituted the majority of the total. The apples were packed in boxes without wraps or shredded paper. In a preliminary examination on March 3, 1943, while the apples were still in storage at 31° F., no scald was found. On April 21 the apples were placed at 70° for 1 week in order to allow any potential scald injury to become manifest. Final inspection on April 28 revealed that ozone had reduced the prevalence and severity of scald.

When the apples were exposed to 3.25 p. p. m. of ozone for the entire storage season 20.5 percent of the red fruit and 9.1 percent of the green became scalded (table 9). In contrast, 76.7 percent of the red fruit and 85.6 percent of the green in the nonozonized lots became scalded. These results were surprising because ordinarily the green skin is more susceptible to scald than the red skin. Severity of the scald was likewise greater in the nonozonized lots, a higher percentage of the fruit being classed as severely scalded than as either slightly or medium, but in the ozonized lots the greater percentages of scalded fruit appeared in the slight and medium classifications. In spite of the reduction of scald in the presence of ozone, however, satisfactory control was not obtained.

TABLE 9.—*Effect of 3.25 p. p. m. of ozone on the development of scald on Arkansas apples*

[Stored at 31° F. until April 21, when placed at 70° for 7 days; examined April 28]

Ozone treatment and description of fruit	Total	Fruit with scald			
		Slight	Medium	Severe	Total
3.25 p. p. m.:	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
Red fruit	146	9. 6	4. 1	6. 8	20. 5
Green fruit	287	2. 1	4. 2	2. 8	9. 1
0 p. p. m.:					
Red fruit	185	22. 2	25. 9	28. 6	76. 7
Green fruit	243	16. 5	28. 0	41. 1	85. 6

None of the other varieties used in these investigations showed any scald development, probably because they were fully mature at time of harvest.

These results are in agreement with those of Smock and Watson (*24*), who found a significant reduction in scald on Rhode Island Greening apples. They stated that ozone did not control scald but reduced its intensity. Earlier investigators (*1*, *2*) reported that ozone has no effect upon scald development.

EFFECT OF OZONE ON QUALITY OF FRUIT

Ozone Injury

No injury resulting from ozone was observed on any of the fruit during the 1941–42 storage season when the ozone concentration averaged 1.95 p. p. m. for the season. However, with the higher concentration of 3.25 p. p. m. of ozone employed during the 1942–43 season, considerable injury occurred and some was noticed on all varieties.

The time of appearance and the extent of the injury varied with the variety, Golden Delicious being the most susceptible and Winesap the least susceptible of the varieties stored. The injury appeared first as slightly discolored spots at the lenticels, indicating killed lenticel tissue. Later these spots became sunken and dark brown. Badly injured fruit was severely pitted and spotted. Most severe injury occurred on fruit nearest the ozonizer and in the surface layers in the packages.

On January 12, the date of the first examination of Golden Delicious apples, which normally have large and conspicuous lenticels, severe injury was found (fig. 7, *A*). As shown in table 10, all of this variety that had been stored in the ozonized atmosphere showed severe injury. Several boxes of Golden Delicious fruit from another storage room without ozone were then placed at various locations in the ozonized room. After 10 days very slight injury was detected on the top fruit near the ozonizer. The injury was conspicuous on Golden Delicious fruit in all boxes after 28 days in storage.

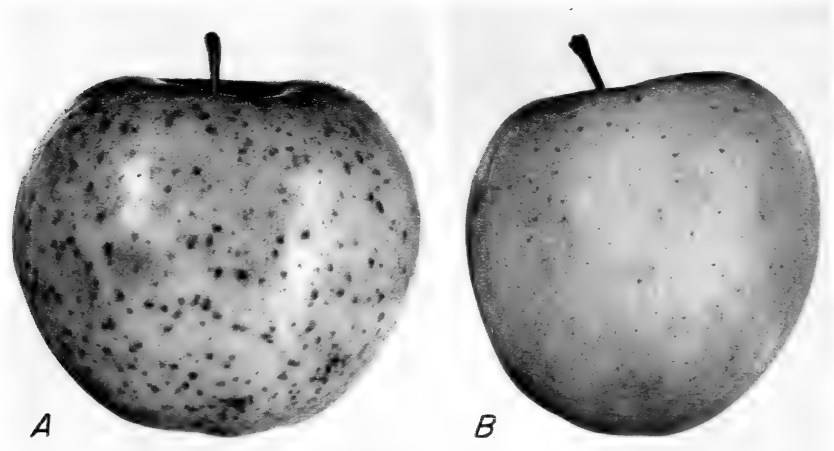

FIGURE 7.—Golden Delicious apples stored 3 months at 31° F.: *A*, Injured by exposure to 3.25 p. p. m. of ozone; *B*, nonozonized.

Corresponding injury was noticed on Rome Beauty apples that were being used for inoculation studies after they had been in the ozonized atmosphere for 30 days. None of the Winesap apples, however, showed any injury upon removal from storage on March 3. On May 24 only slight injury was discernible on 10 percent of the fruit and none was serious enough to be considered objectionable to consumers (table 10). On March 3, 1.3 percent of the Delicious apples were sufficiently damaged to make them objectionable, whereas on April 28, after 7 days at 70° F., 67.8 percent of the red fruit and 17.4 percent of the green fruit of the Arkansas variety were objectionable.

Since the injury just described was produced by long exposure to relatively low ozone concentrations, a test was made to determine whether fruit injury would occur after short exposures to relatively high concentrations of ozone. A box of Rome Beauty apples was placed in an empty storage room and the ozonizer was operated for

TABLE 10.—*Injury to apples stored at 31° F. and exposed to 3.25 p. p. m. of ozone for various periods*

Variety and description of fruit	Date of examination	Length of time in presence of ozone	Fruit injury	
			Slight	Severe
		Days	*Percent*	*Percent*
Golden Delicious	Jan. 12	73	0	100
Delicious	Mar. 3	123	11	1.3
Arkansas:				
Red fruit	Apr. 28 [1]	179	31.5	67.8
Green fruit	Apr. 28 [1]	179	28.6	17.4
Winesap	Mar. 3	119	0	0
	May 24	205	10	0

[1] Held 1 week at 70° F. after removal from storage.

2¾ hours; a concentration of 33.9 p. p. m. was reached. The apples removed from the room the following morning showed no injury. After a few days, however, severe injury was manifest. These results were in sharp contrast with Ewell's statement (*6*) that even 50 p. p. m. has no deleterious effect upon apples. Concentrations of more than 10 p. p. m. for even a relatively short period of storage (a few weeks) might induce injury on some varieties of apples, according to Smock and Watson (*24*) and Watson (*27*). Gane (*12*) found that 11 p. p. m. of ozone for 21 days produced injury on crab apples. It is interesting to note that some investigators found ozone injury on bananas (*11*) after exposure to 1.5 p. p. m. for 10 days and on Hale Haven peaches after exposure to 5 p. p. m. for 2 weeks (*24*).

FLAVOR

No difference was noticed in the flavor of the ozonized and nonozonized fruit of Delicious, York Imperial, and Winesap varieties during the 1941–42 season when the ozone concentration averaged 1.95 p. p. m. The ozonized Golden Delicious apples, however, were superior in flavor to the nonozonized fruit and possessed a more pleasant aroma. No storage flavor was detected in the ozonized lots, whereas it was of frequent occurrence in the nonozonized ones.

However, at the higher concentration of ozone (3.25 p. p. m.) used during the 1942–43 storage season definite impairment of flavor occurred in most of the fruit. A musty flavor which became especially objectionable in the Arkansas and Winesap varieties developed. It was present to a less degree in Delicious and York Imperial also. No objectionable flavor was noticed in Golden Delicious. However, they were removed from storage at the end of their storage season in January and so were not subjected to the ozonized atmosphere as long as were the other varieties.

APPEARANCE

A shiny, sticky, varnishlike cuticle was noticeable on the ozonized Arkansas and Winesap varieties on March 3, 1943, and was very pronounced at the end of the test in late May. This characteristic

was not found on the other varieties, of which ozonized and nonozonized lots were indistinguishable except when ozone injury was noticeable. A similar stickiness of the cuticle as a result of ozone treatment was observed on Rhode Island Greening apples by Smock and Watson (*24*).

Physiological Properties

Apparently ozone has little effect upon the rate of ripening of apples in storage. A number of investigators (*1, 17*) reported that ozone does not accelerate the rate of ripening; and some (*9, 11, 24*) maintained that there is a tendency for ozone to retard the rate because it oxidizes ethylene, which is liberated by the ripening fruit and hastens ripening. Although a slight increase in carbon dioxide production (a measure of respiration) has been reported (*1, 11, 12*), this increase did not result in any hastening of maturity. Pressure tests and chemical analyses by Baker (*1*) showed no differences in hardness or in sugar and starch contents of ozonized and nonozonized fruit. Because of the sticky, varnishlike surface of the ozonized Arkansas and Winesap varieties and its possible effect on the permeability of the cuticle and on the rate of ripening, pH, total-acidity, and internal-atmosphere determinations were made. The differences between the ozonized and nonozonized fruit were small, however, and had no significance.

The findings in these physiological tests are summarized in table 11.

Table 11.—*Effect of 3.25 p. p. m. of ozone for a storage season on some physiological properties of apples*

Variety and ozone treatment	Fruit waxed or unwaxed	Storage period at 31° F.	Subsequent period at 70° F.	pH	Total acidity as malic acid	Internal atmosphere		
						Total gas	Carbon dioxide	Oxygen
		Months	*Days*		*Percent*	*Cubic centimeters per kilogram*	*Percent*	*Percent*
Arkansas:								
3.25 p. p. m.	Unwaxed	5	0	3.63	0.39	306.2	7.3	18.7
			4	3.80	.27	282.0	10.2	13.4
0	do	5	0	3.83	.40	287.0	6.8	18.2
			4	3.96	.30	296.0	12.8	11.3
3.25 p. p. m.	do	7	0	3.63	.38	305.7	3.6	19.8
0	do	7	0	3.70	.36	297.6	4.6	18.6
Winesap:								
3.25 p. p. m.	Waxed	7	2			316.0	10.0	13.6
			7	3.78	.31	301.0	8.6	15.6
0	do	7	2			326.6	11.4	12.2
			7	3.78	.29	328.3	8.0	12.4
3.25 p. p. m.	Unwaxed	7	2			312.6	7.6	16.4
			7	3.78	.30	314.5	8.2	15.2
0	do	7	2			312.6	11.9	12.9
			7	3.73	.31	333.3	8.0	14.4

SUMMARY

The chief values of ozone in apple storage are its maintenance of a pleasant atmosphere in the storage room and the control of surface molds on packages and walls.

Ozone did not control decay of apples, and it did not reduce infection of inoculated wounds. It did, however, retard the rate of enlargement of the infected areas.

Spores of *Penicillium expansum* unprotected by fruit tissue or other organic matter were killed by continuous exposure to ozone. Colonies established on package surfaces, however, were very resistant and were not killed by continuous exposure for 5 months to an atmosphere containing 3.25 p. p. m. of ozone.

Scald was not controlled by ozone, but its development was reduced.

Fruit was injured by a daily exposure to 3.25 p. p. m. of ozone. The exposure period required to produce injury and the degree of injury varied with the variety. No fruit was injured by daily exposure for 5 months to 1.95 p. p. m.

The flavor of all varieties tested except Golden Delicious was impaired by 3.25 p. p. m. of ozone, but no impairment by 1.95 p. p. m. was detected for any variety tested.

The cuticle of some varieties became sticky and varnishlike in the presence of ozone.

No differences in the physiological properties of ozonized and nonozonized fruit were detected.

LITERATURE CITED

(1) Baker, C. E.
1933. effect of ozone upon apples in cold storage. Ice and Refrig. 84: 402–404, illus.

(2) Brooks, C., Cooley, J. S., and Fisher, D. F.
1919. nature and control of apple-scald. Jour. Agr. Res. 18: 211–240, illus.

(3) Dorda, C.
1940. adding ozone to fermentation vats. (to Hefe-Patents G. m. b. H.) Ger. 700,492, Nov. 21, 1940 (Cl. 6a. 15.03). [Abstract in Chem. Abs. 35: 7110. 1941.]

(4) Elford, W. J., and Ende, J. van den.
1942. an investigation of the merits of ozone as an aerial disinfectant. Jour. Hyg. [Cambridge] 42: 240–265, illus.

(5) Ewell, A. W.
1930. the disappearance of szone in cold storage rooms. Refrig. Engin. 20: 358–360, illus.

(6) ———
1936. the use of ozone in cold storage plants. Ice and Refrig. 91: 295–296.

(7) ———
1938. present use and future prospects of ozone in food storage. Food Res. 3: 101–108.

(8) ———
1941. researches on ultra violet light and ozone and their relations as germicidal agents. Refrig. Engin. 41: 331–334, illus.

(9) ———
1942. allies of refrigeration in food preservation. Refrig. Engin. 43: 159–161.

(10) ———
1946. ozone and light: II. ozone. *In* Amer. Soc. Refrig. Engin., The Refrigerating Data Book: Refrigeration Applications Volume. Ed. 2, Sect. II. Cold Storage Practice. Chap. 18, pp. 196–199. Menasha, Wis.

(11) GANE, R.
1935. THE EFFECTS OF OZONE ON BANANAS. [Gt. Brit.] Food Invest. Bd. Rpt. 1934: 128–130.
(12) ———
1936. EFFECT OF OZONE ON FRUIT. [Gt. Brit.] Food Invest. Bd. Rpt. 1935: 126–127, illus.
(13) GOSS, M. J., and PHILLIPS, M.
1938. AN OZONIZER FOR LABORATORY USE. Assoc. Off. Agr. Chem. Jour. 21: 326–331, illus.
(14) HAINES, R. B.
1936. THE EFFECT OF PURE OZONE ON BACTERIA. [Gt. Brit.] Food Invest. Bd. Rpt. 1935: 30–31.
(15) HARTMAN, F. E.
1924. THE INDUSTRIAL APPLICATIONS OF OZONE. Amer. Soc. Heating and Ventilating Engin. Jour. 30: 711–727, illus.
(16) HEILING, A., and SCUPIN, L.
1935. DIE WIRKUNG VON OZON AUF DAS PILZ UND BAKTERIENWACHSTUM IN OBST- UND GEMÜSEKÜHLRÄUMEN; EIN BEITRAG ZUR OZONFRAGE. Ztschr. f. die Gesam. Kälte Indus. 42: 110–116, illus.
(17) HOMAN, C.
1937. EFFECTS OF IONIZED AIR AND OZONE ON PLANTS. Plant Physiol. 12: 957–978, illus.
(18) KAESS, G.
1939. DIE GASKALTLAGERUNG LEICHTVERDERBLICHER LEBENSMITTEL. Angew. Chem. 52: 17–21, illus.
(19) KESSLER, H.
1936. B. KÜHLLAGERUNG MIT OZON. Landw. Jahrb. der Schweiz 50: 598.
(20) KLOTZ, L. J.
1936. NITROGEN TRICHLORIDE AND OTHER GASES AS FUNGICIDES. Hilgardia 10: 27–52, illus.
(21) SCUPIN, L.
1938. A CONTRIBUTION TO THE OZONE QUESTION. Obst u. Gemüsebau 84: 67–68, illus. [Abstract in Rev. Appl. Mycol. 17: 686–687. 1938.]
(22) SMOCK, R. M.
1941. RECENT ADVANCES IN THE STORAGE OF APPLES. N. Y. State Hort. Soc. Proc. 86: 182–183.
(23) ——— and VAN DOREN, A.
1939. STUDIES WITH MODIFIED ATMOSPHERE STORAGE OF APPLES. Refrig. Engin. 38: 163–166, illus.
(24) ——— and WATSON, R. D.
1941. OZONE IN APPLE STORAGE. Refrig. Engin. 42: 97–101, illus.
(25) THORP, C. E.
1940. STARCH-IODIDE METHOD OF OZONE ANALYSIS. Indus. and Engin. Chem., Analyt. Ed. 12: 209.
(26) ———
1941. INFLUENCE OF NITROGEN OXIDES ON THE TOXICITY OF OZONE. Amer. Chem. Soc. News Ed. 19: 686–688, illus.
(27) WATSON, R. D.
1943. SOME FACTORS INFLUENCING THE TOXICITY OF OZONE TO FUNGI IN COLD STORAGE. Refrig. Engin. 46: 103–106, illus.

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